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Comparison of Different Methods for Microencapsulation of Probiotics

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Abstract. At the Department of Food Technology at Fulda University of Applied Sciences different methods for microencapsulation of *Lactobacillus reuteri* DSM 20016 were investigated. The aim of these studies was to develop a process to stabilize the probiotic bacteria for storage and to prevent them from the gastric conditions, to ensure that a satisfactory amount of the probiotics could reach their target location, the human intestine. Drying processes like spray drying and freeze drying were tested as well as fluidized bed granulation with optional Wurster coating using different auxiliary materials. As encapsulation material maltodextrine, sweet whey powder or gummi arabicum were used. The coating experiments were performed with an aqueous shellac solution.

In the performed studies the fluidized bed bottom spray granulation with an additional Wurster coating turned out to be an encouraging procedure to keep the probiotics in a stable form resistant against gastric conditions. The survival rate in the simulated gastro-intestinal passage could be increased up to the sevenfold amount of the untreated bacteria.

Keywords: fluidized bed granulation, Wurster coating, spray drying, freeze drying, *Lactobacillus reuteri*

1. Introduction

Probiotic microorganisms play a major role in today's nutrition. The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) define probiotics as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2001). Various health benefits, for example on disorders relating to the gastro intestinal tract, are reported on the consumption of different probiotics. The report "Health and Nutrition Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria" from FAO/WHO Expert Consultation 2001 gives an extensive overview about the properties of probiotics to the human health (FAO/WHO 2001).

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To get a healthy effect on the consumer of probiotic food, the concentration of the viable microorganisms has to be in a sufficient range. Therefore it is a great challenge to bring the probiotics into a stable form, which guarantees, that the microorganisms reach their target location, the human intestine, in a satisfactory amount. This issue includes on the one hand the problem of storage of the probiotic material itself as well as the storage of the probiotic foodstuff and on the other hand the time-released utilization in the gastrointestinal passage. For use in foods, probiotic microorganisms should not only be capable of surviving passage through the digestive tract but also have the capability to proliferate in the gut. This means they must be resistant to gastric juices and be able to grow in the presence of bile under conditions in the intestines, or be consumed in a food vehicle that allows them to survive passage through the stomach and exposure to bile. They are Gram positive bacteria and are included primarily in two genera, *Lactobacillus* and *Bifidobacterium* (FAO/WHO 2001, Holzapfel et al. 1998; Klein et al. 1998).

Microencapsulation is a suitable technology to prevent the microorganisms from the harmful conditions in the human gastro-intestinal tract and to allow them to develop their healthy effect in the human gut. Various microencapsulation techniques for a broad range of probiotics have been reported. Chávarri et al. and Himanshu et al. (2013) give a detailed review about methods for microencapsulation like extrusion or emulsion techniques as well as drying processes (fluidized bed drying, spray drying or freeze drying) or granulation processes with several carrier materials such as starch, skim milk, alginate, gummi arabicum, κ -carrageenan, chitosan. To protect the probiotic bacteria from thermal injury during the drying process trehalose or sorbitol could be added to the cell suspension as heat protectant (Wassermann et al. 2011) or protection against cold (Azim et al. 2012).

So the aim of different studies, carried out at the Department of Food Technology of the Fulda University of Applied Sciences, was to develop a microencapsulation process for *Lactobacillus reuteri* with an additional coating process to prevent the probiotic microorganisms from the hostile conditions during storage and consumption.

2. Materials and Methods

The studies were performed on *Lactobacillus reuteri* DSM 20016, a probiotic lactic acid bacterium. After (pre-) cultivation of the *L. reuteri* in shaking flask batch slurry fermentation in de Man-Rogosa-Sharpe (MRS) broth the

bacteria were dried in different drying processes and with different additives. Spray-drying processes were conducted with maltodextrine (MD), gummi arabicum (GA) or sweet whey as encapsulation material for the microorganisms. With two of these substrates (MD and GA) freeze drying processes were performed, too. In comparison to these straight drying processes for encapsulation of the *L. reuteri* a granulation process, operated in a fluidized bed granulator with bottom spray, was the topic of additional studies. As encapsulation material here maltodextrine or sweet whey powder was used. Furthermore the received granules were coated in a Wurster process with shellac to improve the resistance against the gastric conditions. Also in a fluidized bed granulation process, but with top spray mode, different additives for the protection of the bacteria against thermal stress during the granulation were investigated. The amount of viable cells in the obtained powders, granules and coated granules as well as in the original fermented slurry, specified in colony forming units per gram (CFU/g) or colony forming units per milliliter (CFU/ml), was evaluated by pour plate count method. Based on these bacterial counts the survival rates for *Lactobacillus reuteri* during the drying, granulation and coating processes and the storage periods could be determined.

The relative viability of the *L. reuteri* after the process was calculated as the bacterial survival rate by the following equation (1) (Schell, D.; Beermann, C. 2014, Nagy, Ádám György 2015):

$$SR = \frac{C1 * m1}{C0 * V0} * 100 \quad (1) \quad (\text{Nagy, Ádám György 2015})$$

where

SR [%]	= bacterial survival rate of the process
C1 [CFU*g ⁻¹]	= viable cell concentration of the product
m1 [g]	= total weight of the product
C0 [CFU*mL ⁻¹]	= viable cell concentration of the slurry
V0 [mL]	= volume of the slurry

The conversion rate of the bacteria describes the relation between the bacteria amounts in the dried product and the bacteria slurry, see equation (2) (Nagy, Ádám György 2015).

$$CR = \frac{C1}{C0} * 100 \quad (2) \quad (\text{Nagy, Ádám György 2015})$$

where

CR [%]	= bacterial conversion rate of the process
C1 [CFU*g ⁻¹]	= viable cell concentration of the product
C0 [CFU*mL ⁻¹]	= viable cell concentration of the slurry

Additionally in this way the amount of viable cells during the simulated gastrointestinal passage and with it the survival and release rate under gastrointestinal conditions was evaluated.

2.1. *Lactobacillus reuteri*

Lactobacillus reuteri is a natural inhabitant of the gastro-intestinal tract of mammals and birds. *L. reuteri* are gram-positive, rod-shaped, heterofermentative lactic acid bacteria. Optimal growth temperature of these anaerob but aerotolerant bacteria is between 30 – 40 (45) °C at a pH-value of 5 – 6. The lethal temperature is reached with 65°C (sub-lethal range 50 – 52°C) (Kandler O.; Stetter K.; Kohl R. 1980, Reuter G. 1965).

The bacterial strain *Lactobacillus reuteri* DSM 20016, used for the performed studies, was obtained from Leibniz-Institut DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany.

2.2. Encapsulation materials

The following substances were used as encapsulation materials: – Spray dried Maltodextrine (DE19) from different suppliers; – Gummi Arabicum powder, spray dried, from Carl Roth GmbH & Co. KG, Karlsruhe, Germany; – Spray dried Sweet Whey Powder Bayolan® from Bayerische Milchindustrie eG, Landshut, Germany

2.3. Coating material

For the coating processes SSB® Aquagold (from Stroeever GmbH & Co. KG, Bremen, Germany), an aqueous shellac solution (25%(w/w)), was used.

2.4. Spray drying

Spray drying is a convective drying process which allows the removing of humidity (solvent, dispersion agent) in one single step. The principle of spray drying is to divide the liquid phase into very small droplets by using suitable atomization devices. This generates a big surface for heat and mass transfer with the drying air. Although high temperatures for the drying air are recommended spray drying is a gentle drying method because of the very short process time.

2.5. Freeze drying

Freeze drying is a drying procedure which removes the humidity out of the frozen state of the product by using vacuum. The drying takes place at pressures and temperatures below the triple point of the solvent. So this method is suitable for temperature sensitive products.

2.6. Fluidized bed granulation

The liquid product (solution, suspension, dispersion) is directly sprayed into a fluidized bed of the carrier material. This encapsulation material is fluidized by (warm) air, which flows through the material from bottom to top. The injection of the liquid by using a two-phase nozzle could take place in the bottom of the fluidized bed or on top of the fluidized bed.

2.7. Wurster coating

A circulating fluidized bed could be generated by the use of a Wurster tube within a fluidized bed granulator. By spraying (bottom spray) a liquid coating material (solution or molten mass) into this circulating fluidized bed the particles could be covered with a homogeneous layer out of the coating material.

3. Results

3.1. Encapsulation

Spray drying is one of the straight drying processes which were investigated for the encapsulation of the microorganisms. Several additives in different concentrations were tested as encapsulation material for the *Lactobacillus reuteri*. The spray drying experiments were executed on a Büchi 190 Mini Spray Dryer (BÜCHI Labortechnik GmbH, Essen, Germany). The bacteria culture of *Lactobacillus reuteri* was suspended in a solution/suspension of maltodextrine (10 or 20%) on the one hand and a solution/suspension of gummi arabicum (5 or 10%) on the other hand. With an inlet temperature of 87°C and the outlet temperature of 55°C for the drying air a survival of the *L. reuteri* could be observed. The bacteria survival rate describes the relation between the amounts of viable cells in the bacteria slurry before the drying and in the rehydrated dry product; the concentration of the dry product in this suspension for determination of the viable cell numbers correspond to the solid content in the bacteria slurry before the drying. The survival rates ranged between 40 and 90% for the maltodextrine as carrier material and between 20 and 50% for the gummi arabicum, Figs 1 and 2. With an increasing outlet temperature up to 62°C (combined with increasing inlet temperature up to 100°C) of the drying air the survival rates decreased significantly down to 4% for MD (10%) as encapsulation material (Szántó, Viola 2013).

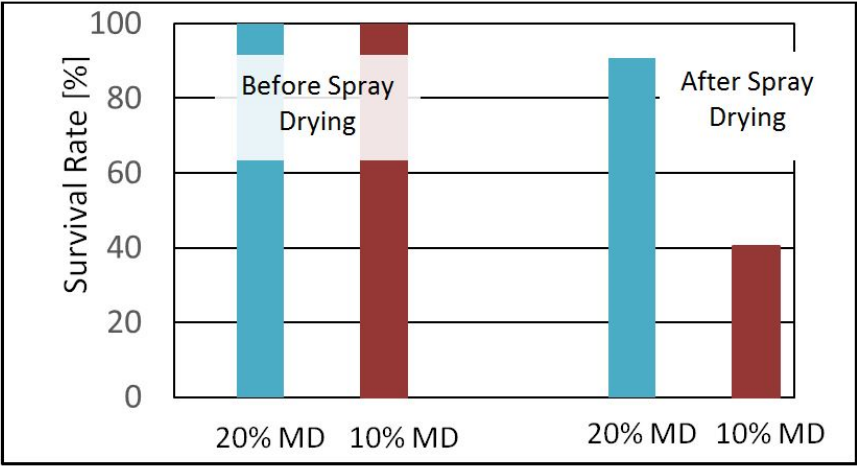


Fig. 1. Bacteria survival rates before and after spray drying with 10 and 20% maltodextrine (MD) as encapsulation material (Szántó, Viola 2013)

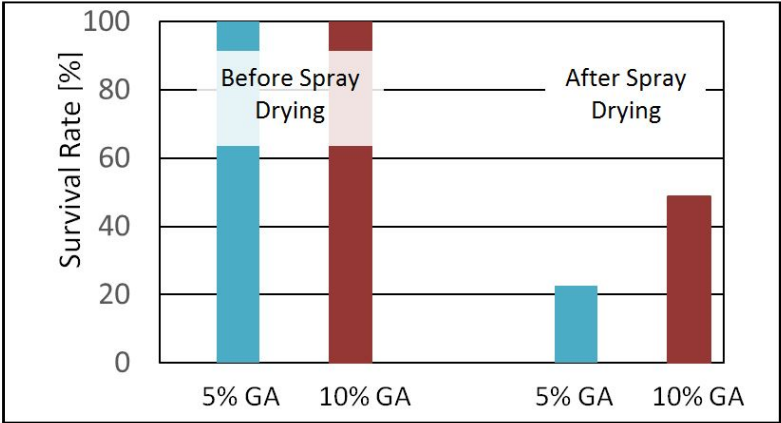


Fig. 2. Bacteria survival rates before and after spray drying with 5 and 10% gummi arabicum (GA) as encapsulation material (Szántó, Viola 2013)

Furthermore a slurry of *L. reuteri*, fermented in 20% sweet whey solution with 0.5% yeast extract as supplement, was dried directly in the Büchi 190 Mini Spray Dryer, too. Independent of the outlet temperature

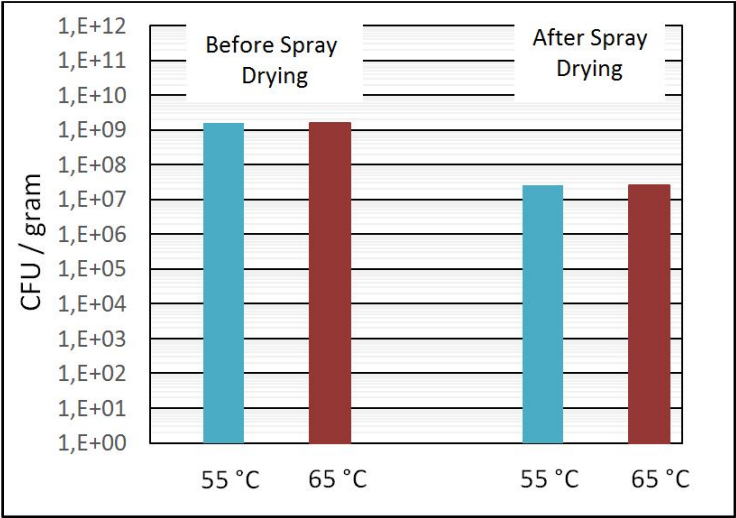


Fig. 3. Cell counts of *L. reuteri* before and after spray drying process with 55°C and 65°C outlet temperature, directly from slurry-fermentation in whey 20% (*w/v*) with 0.5% (*w/v*) yeast extract supplementation. The data shown represents the mean of at least 3 measurements (Jantzen, M.; Göpel, A.; Beermann, C. 2013).

(55°C and 65°C) of the drying air the numbers of bacteria decreased about two orders of magnitude, see Fig. 3. Before the determination of the viable cell amount of the spray dried product, this was rehydrated at a solid content of 20% (*w/v*), according to the solid content in the spraying slurry (Jantzen, M.; Göpel, A.; Beermann, C. 2013). This decreasing of viable cells during the spray drying process comes up to a survival rate of approximately 1.5 %. But nevertheless the total number of viable cells after the spray drying process is still within a satisfactory range.

The second straight drying process which was examined was the freeze drying. The tests were carried out in a freeze dryer Beta 2-16 (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany).

As encapsulation material maltodextrine and gummi arabicum, each in a concentration of 10% and 20%, were used. Figures 4 and 5 show the achieved survival rates for the *L. reuteri* in the conducted freeze drying processes. The survival rates were determined as the relation between the cell counts of the slurry to be dried and in the equal solid content rehydrated dry product. The maximum survival rates were 19% for 10% MD as additive and 10.7% for 10% GA as additive (Szántó, Viola 2013).

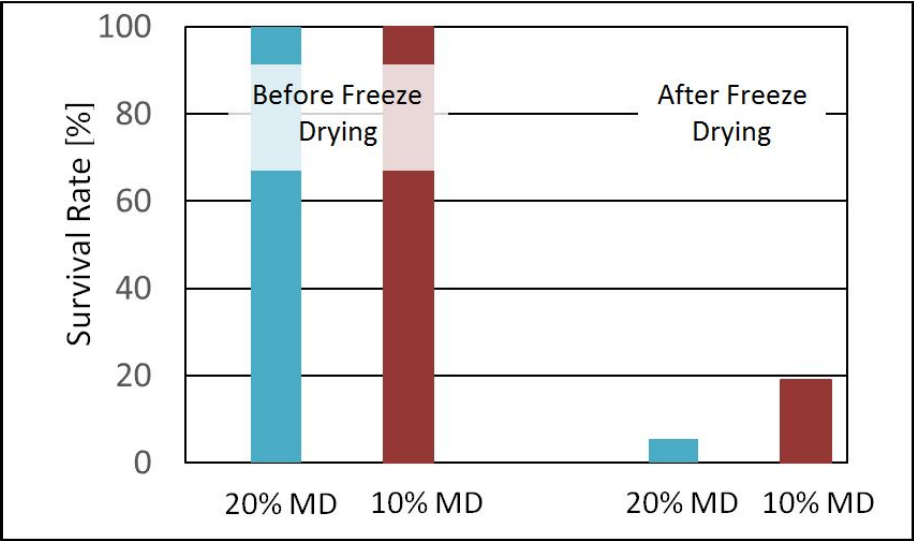


Fig. 4. Survival rates for the *L. reuteri* after a freeze drying process with 10 or 20% maltodextrine (MD) as encapsulation material (Szántó, Viola 2013)

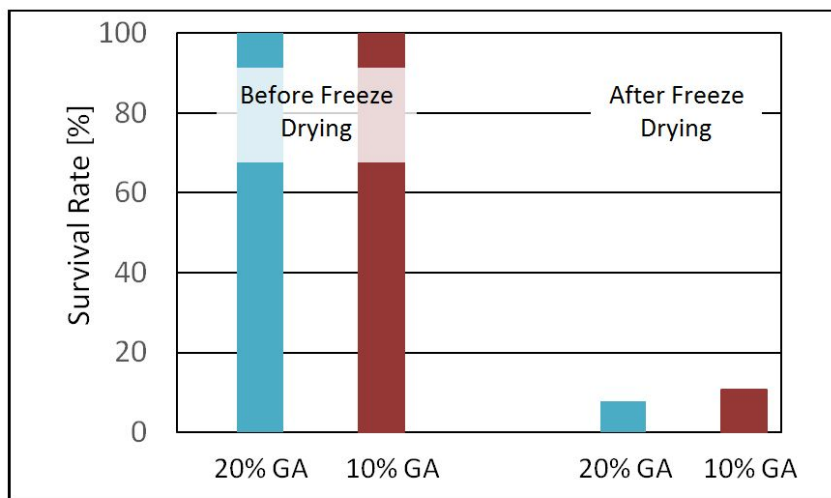


Fig. 5. Survival rates for the *L. reuteri* after a freeze drying process with 10 or 20% gummi arabicum (GA) as encapsulation material (Szántó, Viola 2013)

Besides the straight drying processes the fluidized bed granulation process (FBGP) was examined, too. Two different operation methods were investigated, top spray technique on the one hand by using a Midi Glatt fluidized bed granulator (Glatt GmbH, Binzen, Germany) and on the other hand the bottom spray mode by using a Diosna Minilab fluidized bed granulator (Diosna, Diercks & Söhne GmbH, Osnabrück, Germany). As encapsulation material in these processes maltodextrin was used as well as sweet whey powder. Optionally the granulation process was followed by a coating process with shellac to improve the viability of the dried *L. reuteri*. In the experiments which were operated with the top spray mode of the FBGP sweet whey powder was used as encapsulation material. The granulation experiments were executed at an inlet temperature for the fluidizing air of 50°C, with an atomizing pressure of 0.6 bar for 15 minutes process time. Different extra additives, trehalose, sorbitol and maltodextrine, were tested to protect the bacteria from the thermal impact during the granulation process. The effect of a top spray coating with shellac in a second step after the granulation was tested with this experimental setup, too. As shown in Fig. 6, relative viabilities of nearly 43% could be achieved with trehalose or sorbitol as heat protectant (Schell, D.; Beermann, C. 2014).

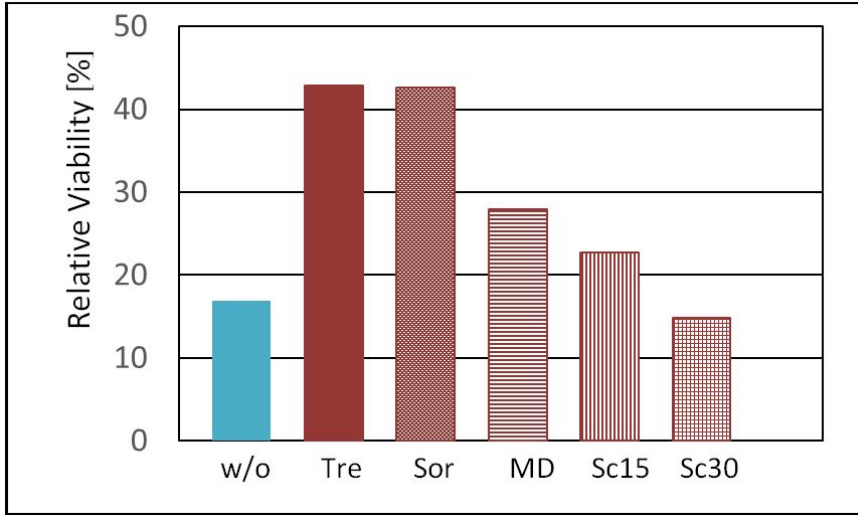


Fig. 6. Observed relative viabilities of *L. reuteri* in absence (*w/o*) and in presence of the thermos-protectants trehalose (Tre), sorbitol (Sor) and maltodextrine (MD) after fluidized bed granulation at 50°C for 15 min, as well as top-spray coating with shellac for 15 min (Sc15) and 30 min (Sc30) (Schell, D.; Beermann, C. 2014)

Fluidized bed granulation at bottom spray mode allows a more robust process. In this case longer granulation times could be reached. Longer process times enable a higher input of the microorganisms into the generated granules, even if the survival rate itself decreases with increasing process time (Nagy, Ádám György 2015). Bottom spray fluidized bed granulation was performed with maltodextrine as well as with sweet whey powder as encapsulation material. The test series operated with maltodextrine showed additionally to the dependency of the bacteria survival from the process time a dependency from the shear stress of the bacteria slurry, expressed by the atomizing pressure of the slurry, too. Due to a temperature sensitivity of the *L. reuteri* found in preliminary tests the granulations were operated at inlet air temperature of 40°C. The bacteria survival rate decreases steadily down to about 60% after a process time of one hour at low shear stress (0.25 bar atomizing pressure). On the other hand a decrease of the survival rate for the *L. reuteri* down to about 30–40% was found for higher shear stress of the bacteria slurry (1 and 2 bar atomizing pressure), Fig. 7 (Nagy, Ádám György 2015).

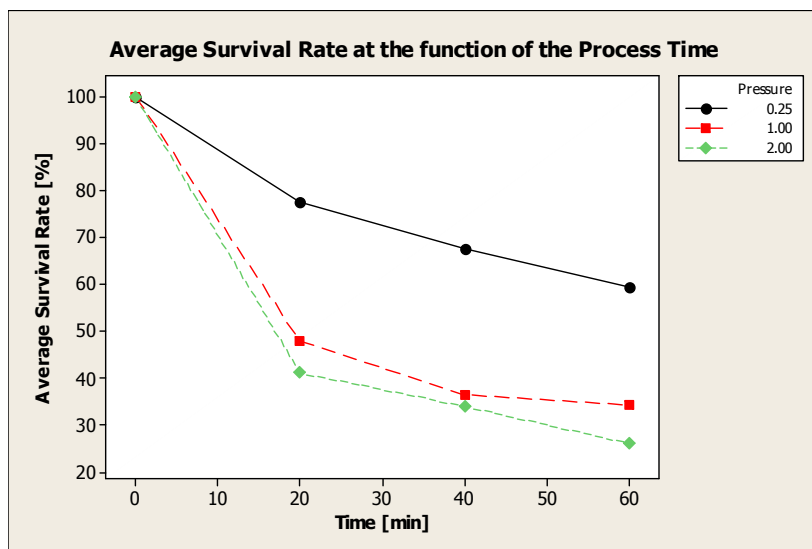


Fig. 7. Average bacterial survival rate as a function of the granulation time with maltodextrine as encapsulation material (Nagy, Ádám György 2015)

In contrast to the straight drying processes the fluidized bed granulation is a batch process in which during the process time continuously more microorganisms could be brought into the product. The decreasing survival rate of the *L. reuteri* with longer granulation times counteracts this advantage. To evaluate the excellence of the process concerning the number of viable microorganisms in the dry product an additional parameter was defined. The conversion rate of the bacteria describes the relation between the bacteria amounts in the dried product and the bacteria slurry (see “materials and methods”, equation (2)) (Nagy, Ádám György 2015).

In Fig. 8 these conversion rates are diagrammed for the granulation of *L. reuteri* with maltodextrine as encapsulation material at an inlet air temperature of 40°C (compare Fig. 7, corresponding survival rates). The bacteria conversion rates increase with increasing process times up to about 75% for an atomizing pressure of 0.25 bar (Nagy, Ádám György 2015).

The effect of coating by using a Wurster device with shellac as coating material in a second step after the granulation was tested with this experimental setup, too. Significant differences in the bacterial survival rate before the coating process and after the coating process could not be determined (Nagy, Ádám György 2015).

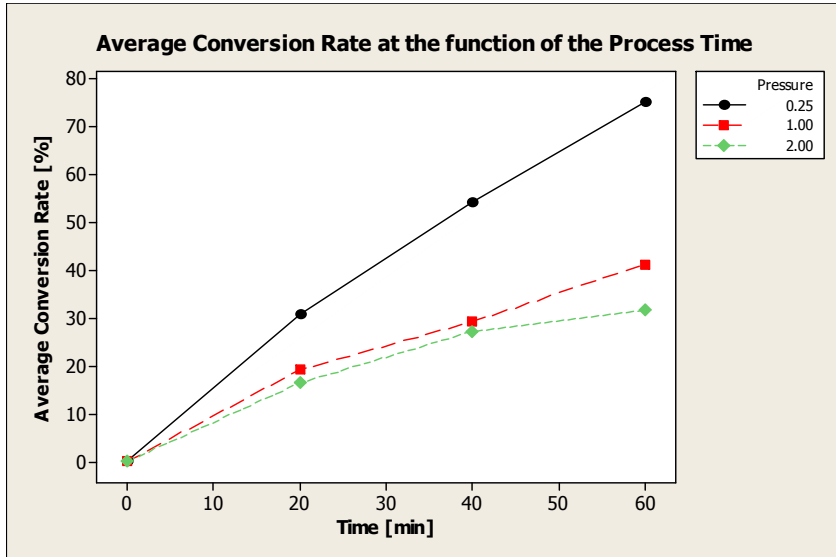


Fig. 8. Average bacterial survival rate as a function of the granulation time with maltodextrine as encapsulation material (Nagy, Ádám György 2015)

In further test series sweet whey powder as encapsulation material was investigated. The experiments were executed with bottom spray fluidized bed granulation followed by a fluidized bed Wurster coating with shellac. A solid granulation process could be achieved, differing to the process with maltodextrine, at an inlet air temperature of 60°C and an atomizing pressure of 2 bar. The granulation time was 60 minutes and the coating time was 60 minutes at 60°C inlet air temperature and 2 bar atomizing pressure, too. First coating samples were taken after 30 minutes coating time. The survival rates of the *L. reuteri* after every single process step are diagrammed in Fig. 9. After the granulation (G) a survival rate of the bacteria of 38% was found. The coating reduced the survival rate down to 14% after 30 minutes (C30) of coating respectively 11% after 60 minutes (C60) (Markó, Lilian 2015).

The relative viabilities shown in Fig. 9 are based on the numbers of colony forming units per milliliter (CFU/ml) specified in Table 1 (Markó, Lilian 2015).

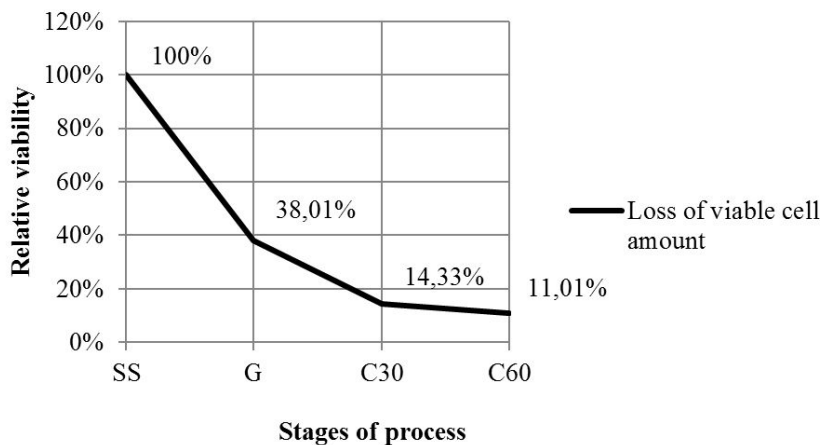


Fig. 9. Relative viability of *L.reuteri* after different stages of microencapsulation (Markó, Lilian 2015)

Table 1. Characteristic of samples in different process stages (Markó, Lilian 2015)

Process	Label	Time [min]	Colony forming unit [CFU/mL]
Bacteria strain preparation	SS	0	$1.96 \cdot 10^8 \pm 3.62 \cdot 10^7$
Granulation	G	60	$7.54 \cdot 10^7 \pm 2.57 \cdot 10^7$
Coating	C30	30	$2.94 \cdot 10^7 \pm 1.05 \cdot 10^7$
	C60	60	$2.21 \cdot 10^7 \pm 8.62 \cdot 10^6$

3.2. Behavior under gastric conditions

The place where the probiotics should develop their positive effects is the human intestine. Therefore the behavior of the microorganisms during the gastrointestinal passage is important for the evaluation of the microencapsulation process for the *L. reuteri*. The microencapsulated product was treated with simulated gastric juice (pH 1.9, containing pepsin) for 30 minutes; then the pH was adjusted to 7.5 and pancreatin and bile salts were added to simulate the intestine juice. At several times in this process samples were taken and the viable cells and from this the survival rates respectively release rates for the bacteria were determined, according to

Picot A., Lacroix C. (2004). Figure 10 shows the survival and release rates under gastric/intestinal conditions for *L. reuteri*, encapsulated with sweet whey powder by spray drying in contrast to not encapsulated bacteria. Under gastric conditions the survival rates drop significantly down to about 10% for the not encapsulated material and about 25% for the encapsulated material. In the further progress of the test the release rate for the *L. reuteri* increases for the encapsulated material to about 50% while the release rate for the not encapsulated material stays at the low level of about 10%. (Jantzen, M.; Göpel, A.; Beermann, C. 2013)

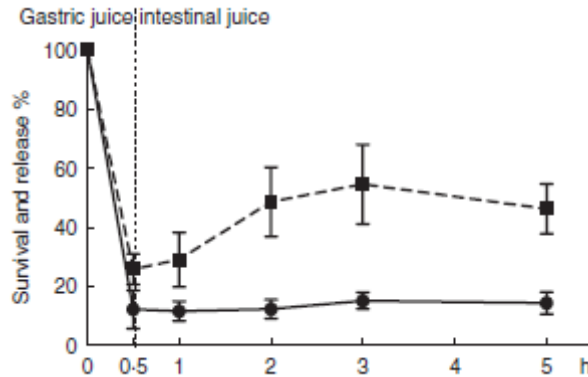


Fig. 10. Percentage release and survival of encapsulated (■) and not encapsulated (●) *L. reuteri* in simulated gastric juice for 30 min and afterwards in simulated intestinal juice for a maximum of 5 h (Jantzen, M.; Göpel, A.; Beermann, C. 2013)

The *L. reuteri* dried in a bottom spray fluidized bed granulation process with maltodextrine as encapsulation material show in an in vitro gastrointestinal test a survival rate of approximately 10% under gastric conditions (see Fig. 11, incubation time half an hour). After the setting up to intestine conditions (increasing pH-value, adding of further digestive enzymes) the number of viable cells and with it the survival/release rate were increasing up to about 200% maximum with the incubation time. Figure 11 presents this for an atomizing pressure of 0.25 bar and different granulation times (Nagy, Ádám György 2015).

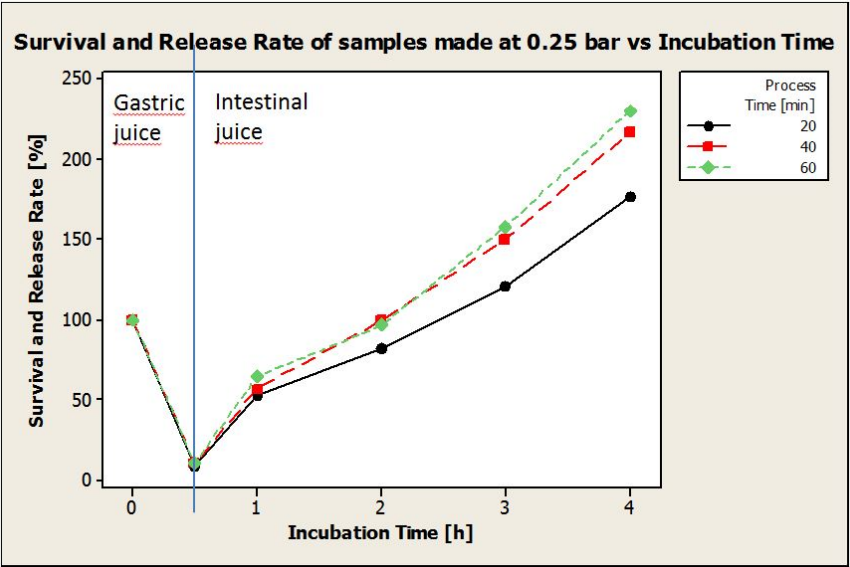


Fig. 11. Survival and release rate of the *L. reuteri* granulated with maltodextrine at 0.25 bar atomizing pressure during incubation in artificial gastrointestinal conditions (Nagy, Ádám György 2015)

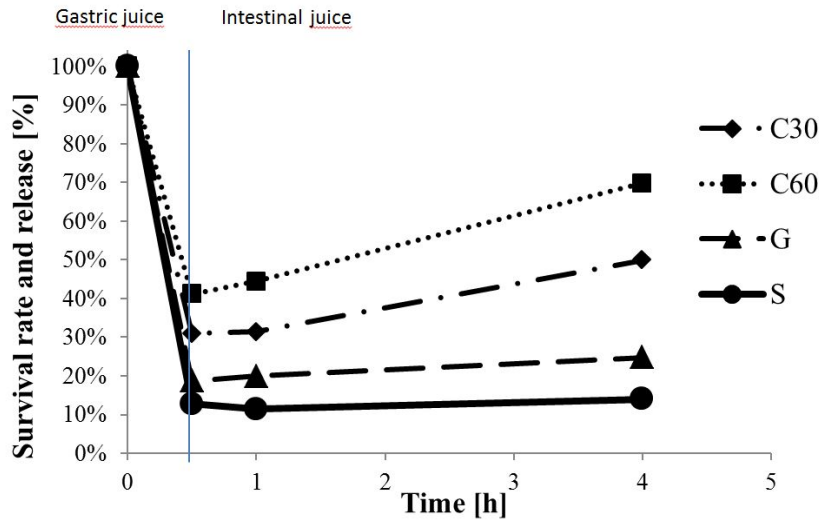


Fig. 12. Survival and release rate of the *L. reuteri* granulated with sweet whey powder (G) and additionally coated with shellac for 30 minutes (C30) respective 60 minutes (C60) in comparison to not granulated/coated material (S) during incubation in artificial gastrointestinal conditions (Markó, Lilian 2015)

Bottom spray fluidized bed granulation of the bacteria with sweet whey powder as encapsulation material followed by a wurster coating with shellac effects an increasing of the survival/release rate of the *L. reuteri* under intestinal conditions, too. After an abrupt drop down after 30 minutes incubation time (according to the gastric conditions) the following intestinal conditions cause for the shellac coated microorganisms a steady rise of the bacteria survival/release rate up to 70% maximum with the incubation time, diagrammed in Fig. 12 (Markó, Lilian 2015).

4. Discussion

In the carried-out studies about microencapsulation of *L. reuteri* the straight drying processes (spray drying and freeze drying) stood vis-à-vis to the granulation processes in a fluidized bed. In every of these processes it was possible to reach cell numbers of the *L. reuteri* in the dried product, which could be sufficient for causing a healthy effect in the human body, even if the microorganisms have to be protected from the conditions during the gastric passage. Anyway the fluidized bed granulation processes seem to be more appropriate for a technical implementation. On the one hand the temperatures of the drying air in the spray drying process have to be in a very low range, so that an efficient operation appears to be problematic. On the other hand the granulation processes give the opportunity to control the reachable viable cell numbers with the process time.

5. Conclusions

The bottom spray fluidized bed granulation process with maltodextrine as encapsulation material followed by an additional coating with shellac in a Wurster process turned out to be a suitable way to bring the *L. reuteri* into a stable form resistant against gastric conditions. In comparison to the fluidized bed granulation with sweet whey powder lower temperatures for the inlet air temperature could be realized, 40°C for maltodextrine compared to 60°C for the sweet whey powder. Shellac turned out to be an appropriate coating material, insoluble in the simulated gastric juice, but able to release the *L. reuteri* in the simulated intestinal juice up to the sevenfold in concentration compared to the uncoated bacteria. To reach the target, namely to provide a sufficient amount of viable *L. reuteri* in the product, which will be released primary in the human intestines, continuing

studies are actually performed at the Department of Food Technology of the Fulda University of Applied Sciences. One focus of these studies is the improvement of the coating process with the aim to generate a homogenous layer around the particles containing the *L. reuteri* with an adequate thickness and without cracks. So that on the one hand the gastric conditions could not harm the bacteria and on the other hand the time-released utilization of the microorganisms could be enabled in the intestinal environment. Another important topic in the further studies is to increase the resistance of the microorganisms against the elevated temperatures (< 60°C) during the encapsulation processes.

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